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Abstract: For deep carious lesions, a more conservative treatment modality ("selective caries removal") has been proposed, where only the heavily contaminated dentine is removed. In this regard, effective adjuncts for cavity disinfection such as the antimicrobial photodynamic therapy (aPDT) can be valuable clinically prior to definitive restoration. Therefore, the aim of this study was to systematically assess clinical studies on the effectiveness of aPDT as a supplementary tool in the treatment of deep caries lesions. Searches were performed in four databases (PubMed, EMBASE, ISI Web of Science, ClinicalTrials.gov) from 1st January, 2011 until 21st June, 2016 for search terms relevant to the observed parameters, pathological condition, intervention and anatomic entity. The pooled information was evaluated according to PRISMA guidelines. At first, 1651 articles were recovered, of which 1249 full-text articles were evaluated, 270 articles thereof were reviewed for eligibility and finally 6 articles met all inclusion criteria. The aPDT protocols involved Methylene Blue, Toluidine Blue and aluminium-chloride-phthalocyanine as photosensitizers and diode lasers, light-emitting diodes and halogen light-sources. The data from five reports, utilizing both culture-dependent and -independent methods, disclosed significant reduction of cariogenic bacterial load after mechanical caries removal with adjunct aPDT. As these studies exhibit some methodological limitations, e.g. lack of positive controls, this systematic review can support the application of aPDT to a limited extent only in terms of reducing the microbial load in deep carious lesions before restorative treatment.

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Antimicrobial photodynamic therapy as an adjunct for treatment of deep carious lesions – a systematic review

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Declaration of interest

Fabian Cieplik declares that he has no conflict of interest.

Wolfgang Buchalla declares that he has no conflict of interest.

Elmar Hellwig declares that he has no conflict of interest.

Ali Al-Ahmad declares that he has no conflict of interest.

Karl-Anton Hiller declares that he has no conflict of interest.

Tim Maisch declares that he has no conflict of interest.

Lamprini Karygianni declares that she has no conflict of interest.

Abstract

For deep carious lesions, a more conservative treatment modality ("selective caries removal") has been proposed, where only the heavily contaminated dentine is removed. In this regard, effective adjuncts for cavity disinfection such as the antimicrobial photodynamic therapy (aPDT) can be valuable clinically prior to definitive restoration. Therefore, the aim of this study was to systematically assess clinical studies on the effectiveness of aPDT as a supplementary tool in the treatment of deep caries lesions. Searches were performed in four databases (PubMed, EMBASE, ISI Web of Science, ClinicalTrials.gov) from 1st January, 2011 until 21st June, 2016 for search terms relevant to the observed parameters, pathological condition, intervention and anatomic entity. The pooled information was evaluated according to PRISMA guidelines. At first, 1,651 articles were recovered, of which 1,249 full-text articles were evaluated, 270 articles thereof were reviewed for eligibility and finally 6 articles met all inclusion criteria. The aPDT protocols involved Methylene Blue, Toluidine Blue and aluminium-chloride-phthalocyanine as photosensitizers and diode lasers, light-emitting diodes and halogen light-sources. The data from five reports, utilizing both culture-dependent and -independent methods, disclosed significant reduction of cariogenic bacterial load after mechanical caries removal with adjunct aPDT. As these studies exhibit some methodological limitations, *e.g.* lack of positive controls, this systematic review can support the application of aPDT to a limited extent only in terms of reducing the microbial load in deep carious lesions before restorative treatment.

Introduction

According to the Global Burden of Disease 2010 study, untreated dental caries in permanent teeth constituted the most prevalent disease across the globe, affecting 2.4 billion people, while untreated dental caries in deciduous teeth was found the tenth-most prevalent condition, affecting 621 million children worldwide [1]. Dental caries is defined as the “localized destruction of susceptible dental hard tissues by acidic by-products from bacterial fermentation of dietary carbohydrates” [2]. Hence, dental caries is considered as a highly dynamic process on the tooth surface with the cariogenic biofilm representing the vital driving force [3].

When the mineral loss has advanced to the point where a cavity forms on the tooth surface, operative dentistry’s role is to restore the structural integrity of the tooth, giving patients the chance to remove/brush off the adherent cariogenic biofilm sufficiently during their daily oral hygiene routine [4]. Traditional treatment concepts for excavation of deep carious lesions suggest the complete and nonselective mechanical removal to hard dentine in order to prevent further cariogenic activity and provide non-demineralized dentine prior to definitive restoration. However, this approach entails the risk of pulp exposure during the excavation process, thus frequently making the application of further therapeutic measures such as pulp capping, pulpotomy or even pulpectomy inevitable [4,5].

To avoid unnecessary vital pulp- or root canal treatment, a more conservative removal of carious dentine (“selective caries removal”) has been proposed, in which only the soft and heavily contaminated (“infected”) dentine is removed, while the demineralized leathery and rarely colonized (“affected”) dentine remains, yielding a cavity ready to be sealed by a definitive restoration [5-7]. This approach is based on the premise that cariogenic bacteria whose carbohydrate supply is cut off either become non-viable or remain quiescent and thus, the caries process is arrested [3,5]. However, although unlikely, it cannot be completely ruled out that remaining bacteria or their metabolites may have any detrimental long-term effects on pulp vitality [7]. Furthermore, distinguishing between the similar-looking layers of dentine from the contaminated or the demineralized zone is quite difficult in most clinical settings [8]. Therefore, effective supplementary approaches for disinfecting remaining bacterially contaminated dentine can be valuable clinically before placing a restoration. Here, the antimicrobial photodynamic therapy (aPDT) may be a promising adjunct for dentine disinfection. Briefly, aPDT involves the application of a

per se non-toxic dye, the so-called photosensitizer (PS), and irradiation with visible light of an appropriate wavelength. Upon irradiation of the PS molecules, either charge (type I) or energy (type II) is transferred to molecular oxygen or other substrates to generate reactive oxygen species (ROS) that kill bacteria via an immediate oxidative burst [9,10]. While oxygen radicals (O_2•-, HO•, H_2O_2) emerge from the type I mechanism, in the type II mechanism an energized molecular oxygen (+0.98 eV) named singlet oxygen is formed and considered to play the major role in photodynamic action; the singlet oxygen quantum yield Φ_Δ describes the proportion of type II mechanism [11].

By applying distinct classes of PS, aPDT has already shown promising results for inactivation of cariogenic bacteria in numerous *in vitro* [12-16] and *in situ* studies [17-20]. Keeping in mind the deficiencies of complete or partial caries removal, the aim of the present review was to systematically assess clinical studies investigating the effectiveness of aPDT as a supplementary modality in the treatment of deep carious lesions.

Methods

Focused question

Is aPDT effective as a supplementary modality in the treatment of deep carious lesions?

Search Strategy

The following electronic databases were screened from 1st January, 2011 until 21st June, 2016 in order to detect eligible papers: PubMed, EMBASE, ISI Web of Science, ClinicalTrials.gov. The search terms for retrieving articles related to dental caries, suitable treatment interventions including aPDT and the evaluation of treatment outcomes with microbiological parameters were divided into four groups:

- **anatomic entity:**

(tooth [Title/Abstract] OR teeth [Title/Abstract] OR dentine [Title/Abstract] OR enamel [Title/Abstract] OR root [Title/Abstract] OR dental hard tissues [Title/Abstract])

- **ethological condition:**

(dental caries [Mesh] OR carious [Title/Abstract] OR tooth decay [Title/Abstract] OR dental disease [Title/Abstract])

- **intervention:**

(prevention OR control OR therapy OR treatment OR microinvasive OR intervention OR inactivation OR eradication OR removal OR management OR medication OR remineralization OR remineralisation OR demineralization OR demineralisation OR modification OR killing OR inhibition OR suppression OR elimination OR reduction OR restoration OR excavation)

- **observed parameters:**

(microbiology OR bacteria OR antibacterial OR antimicrobial OR probiotic OR photodynamic OR microbe OR microbiome OR microbiota OR microorganisms OR oral biofilm OR dental plaque OR streptococci OR streptococcus OR lactobacilli OR lactobacillus OR mono-species OR multi-species OR poly-species OR monospecies OR multispecies OR polyspecies OR polymicrobial OR aerobic OR anaerobic OR dmf OR colony forming units OR CFU OR quantification OR bacterial count).

The grouped listed terms were appropriately combined to yield 16.560 search term matches. Additionally, relevant reviews were also screened for possible literature

matches among their citations, which when considered relevant to the topic, were imported into a mutual EndNote library (EndNote, Thomson Reuters, Toronto, Canada) for all of the screened databases and electronic journals. Finally, all duplicates were automatically discarded from EndNote yielding the total number of relevant articles to be searched.

Inclusion Criteria

In this systematic review the term antimicrobial photodynamic therapy (aPDT) is used to summarize all relevant, mostly non-invasive bacteria-targeting photochemical techniques against cariogenic bacteria with or without the application of a non-toxic local PS in the framework of diverse chairside therapeutic protocols. Therefore, only clinical studies investigating the *in vivo* effect of aPDT on caries-related oral microorganisms in dentine caries lesions in children and adults were taken into consideration. Since aPDT cannot replace but only supplement the routine dental therapy, reports allowing for a co-intervention between aPDT and mechanical caries removal were taken into account. Studies published in both English and German were included.

Exclusion Criteria

Epidemiological reports and systematic or non-systematic reviews were excluded from this study. Studies filtered out of this review mainly comprised *in vitro* reports, as well as all other types of experimental and histological studies. Reports not associated with cariogenic bacteria or involving periodontal or endodontic bacteria were omitted from this review. Moreover, studies on the impact of various preventive or therapeutic interventions against cariogenic microorganisms such as the use of chemical agents *e.g.* chlorhexidine, fluoride, xylitol, natural extracts, probiotics, mechanical inactivation protocols, and restorative materials were not reviewed.

Study Selection

Two independent examiners (LK, FC) were recruited to conduct the primary literature research utilizing the main search terms. Thereafter, the same authors reevaluated the selected titles and abstracts in a second screening round, in which the studies not adhering to the established eligibility and exclusion principles were

omitted. Subsequently, the remaining reports were introduced into a third screening round, in which the full-text articles were further appraised for compatibility. In case of any disagreement between the examiners after independent evaluation, consensus was reached by reevaluation and discussion. The remaining studies were finally introduced into the final review step of qualitative synthesis.

Data Organization

To systematize the data yielded from each report, a standard document was utilized. This document contained year of publication, study design, number of participants, treatment groups, type of intervention, technical parameters of aPDT (light source, peak wavelength, diameter of optical fiber, power output, energy fluence), PS concentration, (pre)irradiation period, methodological aspects such as study design and measurement methods, types of oral microorganisms tested, clinical indices, main outcomes, conclusions and limitations. The dental libraries of the Universities of Freiburg and Regensburg as well as all other contributing authors expertizing in the scientific fields of cariology and aPDT were asked for further interpretation of the collected data when necessary. Additionally, the source reports were evaluated once more in order to guarantee the validity of the yielded data. Due to the small number of the selected reports, no further classification was required.

Data Quality Evaluation

The guidelines of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA, http://www.prisma-statement.org/statement.htm) were followed for the evaluation of the yielded data [21]. The systemization of the obtained data and quality assessment were conducted by two independent examiners (LK, FC) to minimize inconsistencies.

Results

Description of selected studies

Figure 1 shows an overview of the steps followed in the study selection process. After running searches through two English databases and the electronic archives of five German journals, a total number of 1,651 relevant articles could be detected. Following the removal of duplicates 1,249 articles were screened by title and abstract, and 270 full-text articles were further assessed for eligibility after the exclusion of a total of 979 non-eligible articles. The review process then proceeded to further exclude 264 full-text articles for not meeting the prerequisites for inclusion. Finally, seven eligible studies in English language published from 1st January, 2011 until the 21st June, 2016 were selected for the final review [14,22-26]. One study consequently had to be excluded due to the inadequate description of microbiological sampling procedures [22]. Summarized information on the six remaining reports with regard to study design, treatment protocols, clinical and technical parameters, PS, laboratory assays, main outcomes and conclusions are listed in Tables 1-3. The selected reports described the *in vivo* treatment of carious dentine lesions in the primary and permanent dentition using diverse aPDT protocols with combinations of different PS and light sources.

Treatment design and caries removal protocols

In total, six *in vivo* studies were included in this review. In five of these reports aPDT was used to aid the treatment of occlusal dentine caries lesions (class I) in primary and permanent molars of children [14,23,25-27], while two studies involved supportive use of aPDT in treatment of dentine caries lesions (class I, class II) in permanent teeth of adults [24,27]. The number of aPDT-treated teeth in all studies ranged between 10 – 32 among child and 12 – 90 among adult patients. The cavity preparation initially involved the removal of dentine from the lateral walls of carious lesions, which was performed with high-speed hand-pieces and burs [24,25] or excavators [14,23,27]. Thereafter, the removal of carious dentine from the pulp walls was conducted with low-speed carbide burs [23-25] or excavators [26,27]. The collection of the remaining carious dentine from the pulp wall was done prior to (untreated control groups) and after the application of the respective aPDT protocol (test groups) by using micro-punches [23,26], excavators [14,24,27] or low-speed carbide burs [25].

aPDT clinical protocols

With reference to the light-sources, in four studies [23,25-27] aPDT was performed by diode lasers with wavelengths ranging between 630 and 660 nm, two reports [24,26] described the use of light-emitting diodes (LED) at a peak wavelength of 630 nm, while in one study halogen curing light with a wavelength range of 500 – 800 nm was utilized [14].

Methylene Blue (MB, 100 µg/ml) [14,23,25,26], Toluidine Blue (TBO, 100 µg/ml) [24,26] and aluminium-chloride-phthalocyanine (AlClPc, 100 µg/ml) [27] were used as PS. Most commonly, laser irradiation was combined with MB, whereas LED irradiation followed the incubation of dentine in TBO. In all studies the incubation period of the PS in carious dentine ranged between 1 min and 5 min, the irradiation period varied between 1 min and 3 min, while carious dentine was irradiated from distances ranging from 0.5 to 25 mm.

Microbiological outcomes

To investigate the dentine samples obtained prior to (untreated control groups) and after aPDT (test groups), the culture method was applied in four studies [23-25,27] and real-time PCR was applied in one study [26], while one report employed both methods [14]. In five studies [14,23,24,26,27] mechanical caries removal with adjunct aPDT was found to significantly reduce the cariogenic bacterial load and thus to be a potent treatment modality for deep dentine caries. In particular, aPDT therapy yielded a CFU reduction in the range of 0.91 – 2.5 $\log_{10}$ for total viable bacteria, 0.5 – 2.4 $\log_{10}$ for streptococci, and 0.93 – 2.5 $\log_{10}$ for *Lactobacillus* spp. Real-time PCR confirmed these results in one study [26], while in another report [14] no differences could be detected between the control and the aPDT-treated dentine following *S. mutans* DNA quantification. However, in one report [25] aPDT was ineffective at eradicating the viable cariogenic bacteria. Interestingly, one report comparing two different aPDT protocols (LED irradiation + TBO; laser irradiation + MB) disclosed comparable therapeutic outcomes [26].

Discussion

In recent times, there has been a paradigm shift with respect to the controversy, as to how much of the carious tissue in deep dentine lesions has to be removed before placing a restoration [3]. In this regard, effective adjunct approaches for the disinfection of remaining bacterially contaminated carious dentine, such as aPDT may be of great clinical significance. Therefore, a systematic review of clinical studies evaluating the effect of aPDT in deep carious lesions was performed yielding six eligible studies. As a result, aPDT was shown to be effective in reducing the microbial load during restorative treatment of deep carious lesions. However, more clinical trials are required to ensure that this reduction is clinically relevant or not.

At first glance, the fact that all reports considered in this review come from Brazilian groups seems to be a major drawback possibly implying a potential bias. However, it has to be kept in mind that aPDT and even more so anti-cancer photodynamic therapy represent a current research focus in Brazil and are extensively funded by the Brazilian government [28].

In all reviewed studies, the allocation of patients to treatment groups was random. However, blinding the operators is hardly possible in studies involving aPDT due to the necessary application of PS and irradiation with light. Nevertheless, the microbiological analyses of the samples were blinded in all cases. In four out of six studies the traditional culture method was applied for microbial analysis [23-25,27] and in one study real-time PCR was employed [26], while in one study both methods were used [14]. Here, it has to be considered that the culture method measures the viability of cultivable bacterial cells, while real time-PCR is not able to discern between live and dead cells and just measures the amount of DNA belonging to both cultivable and non-cultivable bacterial cells [29]. In that context, it is hardly surprising that Araujo *et al.* did not manage to confirm the significant post-aPDT CFU reduction by applying real time-PCR [14]. On the contrary, the fact that Steiner-Oliveira *et al.* found a significant decline in total bacterial DNA content following aPDT may suggest that an oxidation of nucleic acids occurred in this case [26].

The role of PS is of great importance for the bactericidal outcome of aPDT. Interestingly, five out of six studies outlined in this review used phenothiazinium derivatives (MB, TBO) as PS [14,23-26]. Phenothiazinium dyes show a strong

absorption in the red spectral region (≈ 600 – 680nm) [30]. The remaining study employed aluminium-chloride-phthalocyanine (AlClPc) as PS [27], which exhibits high absorbance in the red spectral region (≈ 650 – 680 nm), too [31]. AlClPc is highly hydrophobic, which is why this PS has to be associated to drug delivery systems for clinical application evolving among others its encapsulation in cationic liposomes as described in the relevant study [27]. The strong blue color of the aforementioned PS may be a drawback for their application in the treatment of dentine caries lesions. In particular, the PS molecules can easily diffuse into dentinal tubules, inevitably leading to a persistent staining of the dentinal structure, which then necessitates a further discoloration treatment [32]. Phenothiazinium derivatives and AlClPc exhibit singlet oxygen quantum yields $\Phi_\Delta \approx 0.5$ or $\Phi_\Delta \approx 0.3$, respectively, thus representing low capacity of singlet oxygen generation compared to other PS classes [33]. As a consequence, the results reported from *in vitro* studies evaluating phenothiazinium derivatives or AlClPc for inactivation of biofilms are quite conflicting [34,35]. Thus, the application of PS with higher singlet oxygen quantum yields like porphyrin derivatives (*e.g.* TMPyP: $\Phi_\Delta \approx 0.74$ [33]) or phenalen-1-one derivatives (*e.g.* SAPYR: $\Phi_\Delta \approx 0.99$ [36]) should be considered for future studies. Indeed, SAPYR was found to be distinctly more effective in inactivation of mono-species biofilms *in vitro* compared to MB when irradiation parameters such as applied light doses or numbers of absorbed photons were adjusted [37].

In general, the inhomogeneous tubular, moist and organic substrate of carious dentine makes the bacterial inactivation by aPDT quite challenging, since sufficient penetration of PS and light transmission are considered key factors for its antimicrobial effectiveness. With respect to PS penetration, the dentinal fluid flow may hamper the PS penetration into the wet demineralized dentine. In recent *in vitro* studies the penetration depths ranged from 45 – 60 µm for MB as measured by Raman spectroscopy [38] to 190 µm for TBO as measured by photoacoustic spectroscopy [39]. While penetration depths of approximately 200 µm are found for oral streptococci in sound dentine [40], the depth of bacterial penetration can be considerably higher in carious dentine [41]. Therefore, improving the PS diffusion rates through dental tissues could be achieved by employing carrier systems for reducing dentinal surface tension or by the introduction of amphiphilic PS that act as detergents [36].

Sufficient light propagation is another key factor for effective application of aPDT in deep carious lesions. It was reported that the irradiance of two given laser light sources was reduced by more than 50% when 150 µm demineralized dentine sections had been interposed, whereby the extent of dentine demineralization had no influence on the aPDT-light distribution [42]. However, our group has recently shown that intra-canal PS could be activated effectively enough from outside the tooth to reach a killing efficacy of 5 $\log_{10}$ steps against *Enterococcus faecalis* [43]. Interestingly, for sound dentine not merely the dentine thickness, but the direction of its tubules seems to have a major impact on light penetration due to multiple scattering caused by the cylindrical microstructure of the dentinal tubules [44,45]. This is the reason why light transmission is hampered by the irregular carious dentinal structure, evolving the presence of some amount of organic and anorganic material in the dentinal tubules which is produced during the biofilm-driven demineralization process. Overall, it is well known that activation of a given PS by red light is favorable since light from longer wavelengths accomplishes greater depth of penetration than short-wave light [46]. Thereby, the irradiation-related temperature changes seem to be negligible with regard to their effect on pulp vitality. Recent studies demonstrated a maximum increase of only 1°C in intrapulpal temperature after aPDT, while a temperature rise of 3°C is considered the safety limit for pulp injury [47,48].

Besides that, the effect of aPDT on dental pulp cells is of pronounced importance, particularly in areas with thin residual dentine layers. Diniz *et al.* observed no reduction in cell viability of dental pulp cells after the application of an aPDT protocol (MB, red laser) in an artificial pulp chamber, where dentine slides with a thickness range from 0.5 to 1.5 mm simulated the pulp chamber roof [49]. Likewise, Longo *et al.* reported no decline in the cell viability of primary human dental pulp cells after their direct exposure to AlClPc-cationic liposomes and irradiation with red laser [27]. Nevertheless, when aPDT (MB, red laser) was directly applied to primary human pulp cell cultures, cell death rates rose proportionally with increased MB concentrations [50]. Surprisingly, while apoptosis remained stable in all aPDT-treated groups, a notably increased amount of necrotic pulp cells was recovered. Consequently, the authors suggested that post-aPDT necrosis in superficial dental pulp tissue might occur *in vivo* as well, potentially leading to the desirable response of mineralization nucleation and the subsequent formation of tertiary dentine [50].

In a recent systematic review investigating aPDT for microbial reduction in deep carious lesions, the authors stated that “aPDT is an effective coadjuvant therapy to reduce microorganisms in deep carious lesions” [51]. According to the data summarized in this review, we unfortunately cannot agree with these conclusions. Although aPDT was effective in reducing the microbiological load in the treated deep carious lesions in five out of six reviewed reports, the overall lack of a positive control group for cavity disinfection is their major shortcoming and severely interferes with their clinical impact. Consequently, it cannot be investigated from the literature whether aPDT may be more effective than standard protocols for cavity disinfection involving the application of chlorhexidine (CHX) in various concentrations. For instance, Wicht *et al.* applied a 1% CHX- and 1% thymol-containing varnish (Cervitec, Ivoclar Vivadent, Schaan, Liechtenstein) on the cavity floors of deep carious lesions, upon their atraumatic restorative treatment (ART), and finally restored them with a compomer (Dyract AP, Dentsply DeTrey, Konstanz, Germany) [52]. After an exposure period of 6 weeks to the CHX- and thymol-containing varnish, microbiological samples exhibited a reduction in microbiological counts by about 1.5 $\log_{10}$ steps. This decline is similar to those that were achieved by aPDT, although in the reviewed studies this was achieved within a notably shorter treatment time period in the range of 1 to 5 min. However, it has to be questioned, whether a microbial reduction of about 1 to 2 $\log_{10}$ steps has any meaningful clinical relevance, as the American Society of Microbiology (ASM) has determined in 2010 that a CFU-reduction of 3 $\log_{10}$ is necessary to use the terms “antimicrobial” or “antibacterial.”

In general, the depth effect of given antimicrobial procedures may be questionable in some part because all of the published microbiological sampling procedures comprise removal of superficial dentine only and subsequent CFU-assay or PCR-analysis from the collected dentinal shavings and (little is known about the penetration properties of given antimicrobials (*e.g.* CHX) in carious dentine. Therefore, this point has to be investigated in future studies. In this regard, novel PS based on a phenalen-1-one that have already shown their detergent potential may be auspicious [36].

Conclusion

Until now, only a few studies on the adjunctive use of aPDT during treatment of deep carious lesions are available. These studies exhibit some methodological limitations, *e.g.* lack of positive controls. Therefore, this systematic review can only support the application of aPDT to a limited extent as an adjunct for the treatment of deep carious lesions in terms of reducing the microbial load in carious lesions before placement of a restoration.

To confirm this assumption more clinical trials are required. In particular, future reports should aim at comparing aPDT directly with standard techniques for cavity disinfection in order to provide useful data for the clinically-relevant evaluation of promising aPDT protocols compared to conventional approaches. Furthermore, the penetration properties of given antimicrobials throughout carious dentine and their depth effects have to be investigated in future studies.

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Author contributions

Conception and design of the experiments: FC, WB, EH, LK.

Literature search: FC, LK.

Data Analysis: FC, WB, KAH, TM, LK.

Authors of the paper: FC, WB, KAH, TM, AAA, EH, LK.

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Table legends

Table 1

Overview of the authors, study design, number of subjects and treated teeth, type of treatment, treatment groups, major outcomes and conclusions of the reviewed studies on photodynamic therapy of cariogenic bacteria.

Table 2

Overview of the used PS, the light sources and the technical features with reference to the aPDT devices as described in the reviewed studies.

Table 3

Overview of the number and type of treated teeth, cavity class and depth, International Caries Detection and Assessment System (ICDAS) index, pulpal involvement, symptoms and exclusion criteria as described in the reviewed *ex vivo* studies on photodynamic therapy of cariogenic bacteria.

Figure legends

Figure 1

Flowchart of the search strategy as well as study selection and data management procedure

Table 1

Authors **Year**	**Study design**	**Number of subjects / treated teeth**	**Treatment groups / Treatment type**	**Microbiological methods**	**Main Outcomes**	**Conclusions**
[Guglielmi et al., 2011]	*in vivo*	22 child patient / 26 teeth with carious lesions	Control group: no treatment Intervention group: Treatment with aPDT (laser irradiation + MB)	Culture method	aPDT therapy yielded a significant CFU reduction of 0.91 log_{10} for total viable bacteria, 1.38 log_{10} for mutans streptococci, and 0.93 log_{10} for *Lactobacillus* spp. compared to the control group.	aPDT was effective at reducing the microbial loads and has beneficial clinical potential for the treatment of deep carious lesions.
[Steiner-Oliveira et al., 2015]	*in vivo*	32 child patients / 32 teeth with carious lesions	Control group: Treatment of carious dentine with 2 % CHX Intervention group: Treatment with aPDT (LED irradiation + TBO or laser irradiation + MB)	real-time PCR	With the exception of *Streptococcus sobrinus* the two aPDT therapies induced a significant reduction in total bacterial content, *Streptococcus mutans*, *Lactobacillus casei*, *Fusobacterium nucleatum* and *Atopobium rimae.* No differences were detected between the two aPDT protocols.	The two tested aPDT-therapies may serve as microinvasive strategies for the effective treatment of deep primary caries.
[Araújo et al., 2015]	*in vivo*	10 child patients / 10 molars with deep active carious lesions	Control group: no treatment (superficial and deep dentine) Intervention group: Treatment of superficial and deep dentine with aPDT (halogen irradiation + MB)	Culture method real-time PCR	Superficial dentine, deep dentine directly and non-directly irradiated: aPDT therapy allowed for a significant CFU decrease of 2.5 ± 0.6, 1.9 ± 0.9 and 2.3 ± 0.8 log_{10} for total viable bacteria, 2.4 ± 0.8, 2.2 ± 0.9 and 2.2 ± 0.9 log_{10} for streptococci, and 2.5 ± 0.7, 2.1 ± 1 and 2.0 ± 0.9 log_{10} for *Lactobacillus* spp., respectively, compared to the untreated carious dentine.	Using conventional culture methods, the effectiveness of aPDT against all estimated viable bacteria was confirmed. However, real-time PCR failed to detect differences in regard to *S. mutans* DNA content. The maintenance of superficial dentine had no impact on aPDT outcomes in deep dentine

					Regarding *S. mutans* DNA quantification by real-time PCR, no differences between the control and the aPDT-treated groups were found.	
[Neves et al., 2016]	*in vivo*	19 child patients / 19 molars with active carious lesions	Control group: no treatment Intervention group: Treatment with aPDT (laser irradiation + MB)	Culture method	aPDT therapy resulted in a statistically insignificant CFU reduction of 0.61 log_{10} for total viable bacteria, 0.44 log_{10} for mutans streptococci, and 0.46 log_{10} for *Lactobacillus* spp. compared to the untreated carious lesions.	aPDT was not effective at eliminating the viable cariogenic microorganisms and is therefore clinically irrelevant for caries treatment in deep dentine
[Melo et al., 2015]	*in vivo*	45 adult patients / 90 teeth with carious lesions	Control group: Treatment of carious dentine with 0.89 % NaCl Intervention group: Treatment with aPDT (LED irradiation + TBO)	Culture method	aPDT group showed a significant CFU reduction of 1.07 log_{10} CFU, while the control group showed a CFU decrease of 0.47 log_{10}. After aPDT the bacterial count of lactobacilli and mutans streptococci reached the greatest log_{10} reduction of 1.69 and 0.5 CFU, respectively, compared to the control.	aPDT-treated dentine from deep carious lesions yielded a significant decrease in cariogenic microbial load
[Longo et al., 2012]	*ex vivo / in vivo*	10 adult and child patients / 12 teeth with carious lesions	Control group: no treatment Intervention group: Treatment with aPDT (laser irradiation + AlClPc)	Culture method	After aPDT the bacterial count of total cariogenic bacteria was reduced by 82% compared to the control.	aPDT was effective at reducing the bacterial load and thus allows for the treatment of deep carious lesions.

Table 2

Authors **Year**	**Light source (peak wavelength [nm])**	**PS (concentration [µg/ml])**	**Optical fiber diameter [µm]**	**Power output [mW]**	**Energy fluence [J/cm^2]**	**Pre-irradiation / irradiation period [min]**	**Distance of irradiation [mm]**
[Guglielmi et al., 2011]	low power diode laser(InGaAIP - Indium Gallium Aluminum Phosphide) (630 nm)	MB (Formula & Ação, Sao Paulo, Brazil) (100 µg/ml)	6000 µm	100 mW	320 J/cm^2	5 min / 1.5 min	0.5 mm
[Steiner-Oliveira et al., 2015]	red light-emitting diode (LED, MM Optics,São Carlos-SP, Brazil) (630 nm) / red low power laser (Photon Lase III-DMC, São Carlos, São Paulo, Brazil) (630 nm)	TBO (100 µg/ml) / MB (Chimiolux®-Hyrofarma, BeloHorizonte, Minas Gerais, Brazil) (100 µg/ml)	-	LED: 100 mW Laser: 100 mW	LED: 30 J/cm^2 Laser: 320 J/cm^2	LED: 1 min / 1 min Laser: 5 min / 1.5 min	-
[Araújo et al., 2015]	halogen light curing unit (Curing Light 3M Espe®, 3M Espe, USA) (500-800 nm)	MB (Chimiolux®, Aptivalux®, Belo Horizonte, Brazil) (100 µg/ml)	-	260 mW	-	5 min / 1 min (with an interval of 20 s between two applications of 30 s)	-
[Neves et al., 2016]	low power diode laser(InGaAIP - Indium Gallium Aluminum	MB (Chimiolux®, Aptivalux®, Belo Horizonte, Brazil) (100 µg/ml)	10000 µm	40 mW	120 J/cm^2	5 min / 2 min	25 mm

	Phosphide) (660 nm)						
[Melo et al., 2015]	red light-emitting diode (LED, MM Optics,São Carlos-SP, Brazil) (630 nm)	TBO (Sigma, St. Louis, MO, USA) (100 µg/ml)	6000 µm	150 mW	94 J/cm^2	5 min / -	2 mm
[Longo et al., 2012]	red light-emitting diode (LED, MM Optics,São Carlos-SP, Brazil) (660 nm)	AlClPc (Aldrich Chemical Company, St. Louis, MO, USA) (5 µM)	1200 µm	40 mW	180 J/cm^2	5 min / 3 min	-

TBO: toluidine blue ortho, MB: methylene blue, AlClPc: aluminum-chloride-phthalocyanine

Table 3

Authors Year	Number / type of treated teeth	Cavity class	Cavity depth	ICDAS index	Pulpal involvement / Symptoms	Exclusion criteria
[Gugliel mi et al., 2011]	26 permanent molars	Class I	Deep carious lesions beyond the inner half of dentine	6	No / No	- Use of antibiotics within last 6 months prior to study - Irreversible pulp inflammation
[Steiner-Oliveira et al., 2015]	32 primary molars	Class I	Deep carious lesions extending to 2 / 3 of the inner half of dentine	6	Compatible with reversible pulpitis / No	- Use of antibiotics for medical reasons - Pain / Irreversible pulp inflammation - missed appointments
[Araújo et al., 2015]	10 molars	Class I	Deep carious lesions extending to 2 / 3 of the inner half of dentine	6	No / No	- Proximal carious lesions - Pulpal / periodontal infection - Insufficient crowns
[Neves et al., 2016]	19 molars	Class I	Deep carious lesions extending to the inner half of dentine	6	No / No	- Use of antibiotics within last 3 months prior to study - Systemic diseases - Irreversible pulp inflammation, pain, fistula, periapical lesion
[Melo et al., 2015]	90 posterior teeth	Class I	Bilateral moderate to deep carious lesions extending to 2 / 3 of the inner half of dentine	6	No / No	- Use of antibiotics within last 3 months prior to study - Irreversible pulp inflammation, abscess, fistula, periapical lesion - Pain, periodontal swelling, tooth mobility
[Longo et al., 2012]	12 primary / permanent molars	Class I	Deep carious lesions extending to 2 / 3 of the inner half of dentine	6	No / No	- Irreversible pulp inflammation - Periodontal disease